AD-A262 979

2

13C NMR SPECTRA OF ALLOSTERIC EFFECTORS OF HEMOGLOBIN

Stanislaw Ostrowski,[1] Thomas G. Burke, and Waldemar Priebe
The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, USA [SO, WP] and College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA [TGB]

KEY WORDS : Allosteric effectors of hemoglobin, Urea and thiourea derivatives, ^{13}C NMR, Spectral assignments.

INTRODUCTION

In the last three years many papers dealing with the preparation and biological activity of compounds that act as allosteric effectors of hemoglobin have been published.[2-4] Among the numerous moieties, some urea derivatives were found to be very effective in reducing oxygen affinity of hemoglobin.[2,3] The structures of most of these compounds were confirmed by ^{1}H NMR spectra; however, to our knowledge, ^{13}C NMR spectra have not been published for any of these allosteric modifiers of hemoglobin. We prepared in this series over 30 new urea and thiourea derivatives with the intent of investigating their biological activity.[5] An analysis of ^{13}C NMR spectra revealed high regularity in the chemical shifts of the similar fragments of the structures and revealed signal deviations among structures having different substituents in the aromatic rings.

RESULTS AND DISCUSSION

A simple analysis procedure was used to assign peaks in the spectra. At first, two peaks corresponding to the signals of the aromatic rings A and B were

STATEMENT
public release
Unlimited

93-07620

93 4 12 054

identified. For R = H in all cases the chemical shifts originating from C-4, C-5(5′), C-6(6′), and C-7 were very close or correspondingly identical (e.g., 18 and 20, Table 1), whereas the C(9) - C(14) signals varied, depending on substituents in ring A. A replacement of R - H by the $C(CH_3)_2CO_2H$ group is reflected in the δ values of the C(4) - C(7) signals, and virtually did not change the C(9) - C(14) signals in the respective pairs of compounds (e.g., 13, 14). For this series (R - $C(CH_3)_2CO_2H$, R^1, R^2 - changeable) the very close occurrence of the corresponding C(4) - C(7) chemical shifts was observed as well (e.g., 14 and 28). On this basis the bulk of the signals (spectra recorded with C-H decoupling technique) was assigned to the appropriate carbons in both aromatic rings. Independently, the calculations of δ values from increments[6)] for all aromatic carbon atoms were done. We found good agreement between the chemical shifts calculated from increments and those experimentally determined (e.g., compound 1, calculated: C-4 (149.8 ppm), C-5,5′ (116.0), C-6,6′ (120.0), C-7(132.7), C-9 (141.0), C-10 (133.9), C-11 (130.1), C-12 (117.6), C-13 (145.2), C-14 (113.9), C-11 (130.1), C-12 (117.6), C-13 (145.2), C-14 (113.3); compound 1, experimental data: 152.8, 115.3, 120.4, 131.2, 138.9, 134.0, 130.7, 116.2, 146.1, 113.3 ppm respectively). For some instances a coupling between carbon atoms and fluorine was diagnostic and helpful for correct assignment. In 15 we were left with two unidentified peaks at 106.2 (broader singlet) and 105.7 (d, J = 5 Hz), originating from C-10 and C-14. Singlet 106.2 was assigned to C-10 owing to the absence of meta carbon-fluorine coupling. This lack of a three-bond coupling constant was revealed when any substituent was present in aromatic ring between the respective centers (see 14-18). Ortho interactions deformating the planarity of arene ring (together with substituents) also decreased $^2J_{CF}$ values, especially when the neighboring group (to fluorine) was relatively bulky, e.g., constants of C-8 and C-10 carbons with fluorine in 14.

In some cases a long-range influence of the substituents on the chemical shifts was observed. When R = H was replaced by the $C(CH_3)_2CO_2H$ group, not only the (C-4) and (C-5,5′) were changed, but also (C-7) was shifted downfield by c.a. 3-4 ppm. This change was relatively smaller on (C-6,6′), c.a. 1.5 ppm. In this series the C-5(5′), C-6(6′) carbons were readily identified by their high intensity, but it was

difficult to correctly assign them because of a close appearance of their chemical shifts. For two selected compounds (6, 12) two-dimensional measurements were recorded. In these cases downfield shifted signals corresponded with C-5(5') protons. On this basis we propose to assign (C-5, 5') and (C-6, 6') values for the remaining compounds in this series.

An introduction of an NH_2 group into terminal aromatic ring A causes not only dramatic changes in the neighboring carbon atom chemical shifts. Also were observed, independently which position NH_2 group occupies (compounds: 5, 6, 9, 12, 15), long-range effects on C-8 (small downfield shift), C-7 (downfield shift) and even C-4 (upfield shift -1 ppm). The neighboring amino group also changed the carbon-fluorine coupling, decreasing it up to 25 Hz (compound 15).

Significant changes in chemical shifts were caused by a thiocarbonyl group in the urea bridge. Carbon atoms C-6(6'), C-10, and C-14, situated in the same distance from C=S are shifted downfield by over 5 ppm in 22, 23, 26 as compared with corresponding urea analogues 20, 21, 25. Interestingly, that even five-bond distanced from C=S C-4 and C-12 are moved downfield by over 2 ppm. this influence is relatively smaller on C-7 and C-9.

EXPERIMENTAL

All ^{13}C and two-dimensional NMR measurements were carried out in 5 mm tubes in DMSO-d_6 using a QE-300 spectrometer operating at 75.234 MHz for ^{13}C and a Bruker AM-400 spectrometer operating at 100.623 MHz for ^{13}C and at 400.139 MHz for ^1H. Sample concentrations were approximately 30 mg ml^{-1}. Other experimental data for ^{13}C NMR were: pulse width, 4.0 μs; acquisition time, 0.27 s; flip angle, 90°; and spectral width 20 kHz. Corresponding data for ^1H were: pulse width, 8.6 μs; acquisition time, 0.8 s; flip angle, 10°; and spectral width, 5 kHz. All data were referenced to DMSO-d_6 at 39.50 ppm. Synthesis and chemical characterization of the compounds 1-30 and 32-33 will be published elsewhere.[5]. Data for other new compounds were as follows (melting points were uncorrected):

1-(3-Trifluoromethylphenyl)-3-(4-hydroxyphenyl) urea (31).

Compound 31 was prepared according a procedure described in the literature[3] and

resulted in 90% yield, m.p. 202°C ($CHCl_3$/MeOH). For $C_{14}H_{11}F_3N_2O_2$(296.25): Calcd C 56.76, H 3.74, N 9.46. Found C 56.66, H 3.75, N 9.50.

1-(4-Methylthiophenyl)-3-(4-hydroxyphenyl) urea (34). Compound 34 was prepared as above, resulting in 96% yield, m.p. 214°C ($CHCl_3$/MeOH). For $C_{14}H_{14}N_2O_2S$ (274.34): Calcd C 61.29, H 5.14, N 10.21, S 11.69. Found C 61.08, H 5.19, N 10.18, S 11.79.

ACKNOWLEDGMENTS

This work was supported by Office of Naval Research Grant No. N00014-90-J-1648.

References

1. Current address: Institute of Organic Chemistry, Polish Academy of Sciences, 01-224, Warsaw, Poland.
2. I. Lalezari, S. Rahbar, P. Lalezari, G. Fermi, M. F. Perutz, Proc. Natl. Acad. Sci. U.S.A., 85, 6117 (1988).
3. I. Lalezari, P. Lalezari, J. Med. Chem., 32, 2352 (1989). also check Biochem. 29, 1515 (1990)
4. R.S. Randad, M. A. Mahran, A. S. Mehanna, D.J. Abraham, J. Med. Chem., 34, 752 (1991); F.C. Wireko, G.E. Kellogg, D.J. Abraham, ibid., 34, 758 (1991); D.J. Abraham, F.C. Wireko, R.S. Randad, C. Poyart, J. Kister, B. Bohn, J.-F. Liard, and M.P. Kunert, Biochem.,31, 9141 (1992).
5. T.G. Burke, S. Ostrowski, S. Rahbar, and W. Priebe. Pharmaceut. Res. 9: S-74 (1992).
6. P. Clerc, S. Simon, Tables of Spectral Data for Structure Determination of Organic Compounds, edited by F.L. Boschke et al., Springer-Verlag, Berlin, Heildelberg, New York, Tokyo, 1983, p. C120

Data for compounds 1-- 34.

Table 1. ^{13}C NMR ~~assignments of allosteric effectors of hemoglobine~~ (~~all~~ coupling constants J ~~are~~ expressed in hertz [Hz]).

Compound		Carbon No & chemical s~~c~~hifts [ppm]														
R^1,R^2	No	1	2	3,3	4	5,5'	6,6'	7	8	9	10	11	12	13	14	Others
10-CH_3,13-NO_2	1				152.8	115.3	120.4	131.2	152.6	138.9	134.0	130.7	116.2	146.1	113.3	18.1(CH_3)
10-CH_3,13-NO_2	2	175.0	78.7	25.0	150.3	119.9	119.5	133.6	152.4	138.7	134.2	130.9	116.4	146.1	113.5	18.0(CH_3)
11-NO_2	3				152.6	115.2	120.9	130.5	152.9	141.4	111.9	148.1	115.9	129.9	124.1	
11-NO_2	4	175.1	78.7	25.0	150.4	119.9	119.7	133.5	152.5	141.2	112.0	148.1	116.0	129.9	124.1	
11-NH_2	5	176.3	79.3	25.4	150.2	119.6	118.9	134.2	152.8	140.7	103.8	148.8	107.7	128.7	106.2	
11-NH_2 R=$C(CH_3)_2CO_2CH_3$	6	173.8	79.0	24.9	149.4	120.3	119.2	134.8	152.5	140.3	103.7	149.1	108.0	129.0	106.1	52.3(CO_2CH_3)
12-NO_2	7				152.1	115.2	120.9	130.3	153.0	146.6	117.2	125.1	140.7	125.1	117.2	
12-NO_2	8	174.9	78.7	25.0	150.6	119.9	119.7	133.3	152.0	146.5	117.3	125.1	140.9	125.1	117.3	
12-NH_2	9	175.1	78.7	25.0	149.6	119.9	119.1	134.7	153.1	128.8	120.6	114.2	143.7	114.2	120.6	
10-OCH_3,12-NO_2	10				151.9	115.3	120.3	130.5	153.0	136.2	146.8	105.5	140.6	115.9	117.6	
10-OCH_3,12-NO_2	11	175.0	78.7	25.0	150.5	119.8	119.5	133.4	151.8	136.0	146.9	105.5	140.7	116.1	117.6	56.5(OCH_3)
10-OCH_3,12-NH_2	12	175.1	78.8	25.1	149.8	120.0	118.8	134.7	152.9	117.6	149.7	98.0	144.5	105.5	121.4	
11-NO_2,12-F	13				152.7	115.2	121.0	130.4	152.9	136.9	114.1	136.4 J=11	149.3 J=255	118.5 J=22	125.3 J=7	

11-NO_2,12-F	14	175.0	78.8	25.0	150.5	120.0	119.8	133.5	152.6	136.8	114.3	136.5 J=9	149.4 J=255	118.6 J=22	125.5 J=7	
11-NH_2,12-F	15	175.0	78.7	25.0	149.8	119.9	119.2	134.3	152.5	136.1	106.2	136.2 J=15	146.2 J=231	114.5 J=19	105.7 J=5	
10-F,13-NO_2	16				151.9	115.1	120.5	130.0	152.9	128.9 J=15	154.6 J=247	115.5 J=22	117.0 J=9	143.0	114.6	
10-F,13-NO_2	17	175.0	78.7	25.0	150.5	119.8	119.6	133.2	152.0	128.9 J=12	154.9 J=252	116.0 J=22	117.5 J=9	144.0	114.5	
11-Cl,12-F	18				152.8	115.2	120.7	130.7	152.8	137.3	119.2	119.1 J=19	152.1 J=239	116.7 J=22	118.2 J=4	
11-Cl,12-F	19	174.4	78.7	24.8	150.2	120.0	119.6	133.7	152.3	136.8	119.6	118.9 J=16	152.1 J=239	116.3 J=22	118.3	
11-Cl	20				152.6	115.2	120.7	130.7	152.8	141.6	117.4	133.2	121.1	130.2	116.4	
11-Cl	21	175.0	78.7	25.0	150.3	119.8	119.7	133.8	152.5	141.4	117.5	133.2	121.3	130.3	116.5	
11-Cl (X=S)	22				154.9	115.0	126.0	130.1	179.9	141.1	122.8	132.3	123.5	129.5	121.6	
11-Cl (X=S)	23	174.5	78.5	24.9	152.3	118.7	124.9	132.2	179.6	140.9	122.6	132.9	123.6	129.5	121.5	
11-Cl,12-Cl	24				152.8	115.2	121.1	130.1	152.8	139.2	119.4	131.7	123.9	129.7	117.4	
11-Cl,12-Cl	25	175.1	78.7	25.1	150.4	119.9	119.8	133.6	152.4	140.1	119.2	131.0	123.0	130.5	118.2	
11-Cl,12-Cl (X=S)	26	177.7	79.6	25.9	153.4	117.6	125.0	132.2	179.4	140.7	123.8	132.0	130.2	129.9	122.7	
11-CN	27				152.5	115.1	120.8	130.4	152.8	140.8	120.5	111.4	124.7	129.8	122.5	118.6(CN)
11-CN	28	175.3	78.8	25.2	150.5	119.9	119.7	133.7	152.7	140.9	120.7	111.7	125.2	130.2	122.8	119.0(CN)
12-CN	29				152.3	115.3	120.9	130.5	153.0	144.5	117.8	133.2	102.9	133.2	117.8	119.4(CN)